Chemical Standard Operating Procedure

All work involving materials classified as Particularly Hazardous requires the completion of Section 6.

Procedure Name	Gel Electrophoresis				
Procedure Author					
Name of Responsible Person					
Location to be Performed					
Creation Date		**Review Date(s)**		**Revision Date(s)**	

1. THIS STANDARD OPERATING PROCEDURE (SOP) IS FOR A:

☐Specific laboratory procedure or experiment
- Examples: synthesis of chemiluminescent esters

☒Generic laboratory procedure that covers several chemicals
- Examples: distillation, chromatography

☐Generic use of a specific chemical or class of chemicals with similar hazards
- Examples: Organic azides, mineral acids, hydrofluoric acid

2. DESCRIPTION: *Briefly describe how the chemical will be used.*

This standard operating procedure outlines the process of electrophoresis, including the use of equipment and chemicals necessary for the process. Review this document and supply the information required in order to make it specific to your laboratory. Electrophoresis uses electrical energy to separate molecules based on their size, structure, and electrical charge.

3. RISK IDENTIFICATION: *Identify potential safety hazards – refer to Section 2 of the SDS.*

☐Explosive	☐Carcinogen
☐Pyrophoric	☐Sensitizer (respiratory and/or skin)
☒Flammable (liquid, solid, gas or aerosol)	☐Irritant (skin and/or eye)
☐Self-Reactive	☒Corrosive (skin and/or eye damage)
☐Peroxide Forming	☒Acute Toxicity (oral, dermal and/or inhalation)
☐Organic Peroxide	☒Germ Cell Mutagen
☐Oxidizing (liquid, solid or gas)	☒Reproductive Toxicity
☐Water-Reactive	☒Target Organ Systemic Toxicity: Single Exposure
☐Compressed Gases	☐Target Organ Systemic Toxicity: Repeated Exposure
☐Cryogen	☐Other: Click or tap here to enter text.
☐Corrosion to Metals	
☐Radionuclides	
☐Other: Click or tap here to enter text.	

Notes (include chemicals that will be used, additional cautions, permissible exposure limits, etc.):

	Hazardous chemicals that may be used (select applicable to your work):
	• Glacial acetic acid
	  ○ Flammable liquid and vapor, causes severe skin burns and eye damage, may be harmful if swallowed, toxic if inhaled.
	• Na2EDTA
	  ○ Causes serious eye irritation, harmful if inhaled, may cause damage to organs through prolonged or repeated exposure
	• Boric acid (particularly hazardous chemical)*
	  ○ May damage fertility or the unborn child. ACGIH TWA = 1 ppm
	• Ethidium bromide (particularly hazardous chemical)*
	  ○ Harmful if swallowed, fatal if inhaled, suspected of causing genetic defects
	
	*In identifying PHC for a laboratory, it is necessary to consider the nature of the hazard. Check SDS – hazardous characteristics may vary based on form and concentration

4.	**WHAT ENGINEERING CONTROLS WILL BE USED TO MINIMIZE EXPOSURES TO THESE HAZARDS?** *select all that apply*
	☒Fume Hood ☐Snorkel ☐Glove Box ☐Clean Room ☐Explosion Shielding ☐Splash Shielding ☐Beta Shielding ☐Safety Storage Cabinet ☐Flammable Storage Refrigerator ☐Other: Click or tap here to enter text.
5.	**WHAT PERSONAL PROTECTIVE EQUIPMENT IS REQUIRED TO MINIMIZE THESE HAZARDS?** *select all that apply*
	☒Safety Glasses ☐Lab Coat ☒Fire-Resistant Lab Coat ☒Gloves - specify type: Kimberly Clark or Microflex Nitrile ☐Acid Resistant Gloves ☐Acid Resistant Apron ☐Face shield ☐Other: Click or tap here to enter text.
6.	**STEP-BY-STEP OPERATING PROCEDURE** *Provide a sequential description of work, including as much detail as possible such as designated work area(s), chemical concentrations ranges and amount used (mass, volume) and when special safety equipment is to be utilized. Include temperature, pressure, and other*

experimental conditions if possible. Pictures and schematics are recommended for complex setups. ***Highlight the steps with the highest hazards.***

Preparing XXX Buffer
(specify designated area if using boric acid or ay other particularly hazardous chemical)

Buffer Selection:
XXX
Buffer Recipe:
XXX – #x stock solution, pH ~#

-
-

Preparing a Gel
An increased % in the gel matrix gives better separation of small fragments and bands that are close in size. *For example*:

Agarose % (w/v)	Approximate range of separated DNA fragments (kb)
0.3	60 to 5
0.5	30 to 1
0.7	12 to 0.8
1.0	10 to 0.5
1.2	7 to 0.3
1.5	4 to 0.2
2.0	3 to 0.1
3.0	< 0.1

For #% Gel:

1. Dissolve # g of XX in # mL of buffer by heating in a microwave.

   CAUTION HOT! USE GLOVES WHEN REMOVING

2. Once the matrix has completely dissolved, and a molecular dye will be used in the gel (i.e. Ethidium Bromide, SYBR Safe, etc), pipette # µL (dilution of 1:#) of XX into the liquid and mix by gentle swirling.

   SYBR SAFE is a less hazardous alternative to ethidium bromide (EtBr). Proper PPE and engineering controls must be used when handling EtBr. Specify designated area when using EtBr.

3. Place the gel tray into the gel box so that the gasket (ends with rubber strip) forms a seal against the walls of the gel box make sure to press the gel tray all the way down so that the gel box and gel tray are level.

4. After the gel mix has cooled to 60°C (Higher temps will damage and warp the gel box) pour the mix into the gel tray. Upon pouring the gel mix immediately insert the gel comb with the desired number of teeth/wells.
5. Allow the gel to solidify completely. Then lift the gel tray out of the gel box turn it 90° and replace it into the gel box with the comb closest to the cathode.
   **Note: If using pre-cast gels, please delete steps 3-5.*
6. Pour running buffer into the gel box to fill the chamber and completely submerge the gel (#ml).
7. Carefully remove the comb using a light tapping motion to avoid damage to the wells.

Loading Samples

Please note the maximum volume for a gel of this size using a X comb is #µL
For other combs use the calculation below to determine well volume and x 0.75 of this value for loading volume.

(Well height (mm)-1.5) x (Tooth width x Comb thickness)

1. Pipette #µL of your sample into a clean tube.
2. Add #µL of loading dye (Bromophenol blue) for visual tracking.
3. Mix by low vortex and centrifuge to ensure all sample is at the bottom of the well.
4. Plan out your gel before pipetting and create a reference chart/diagram to ensure correct samples are put into the correct wells. Also ensure that you have a negative and positive control in addition to a molecular weight marker ladder on one or both ends of the gel (first and last well).
5. Carefully pipette all of sample into the correct well on your gel using compatible extended length pipette tips.
6. Add #µL of the molecular weight marker ladder for reference/comparison in at least one, or if possible, both ends of your gel (first and last wells).
7. Load all remaining empty wells with # µL of sample loading buffer to ensure proper and consistent migration of the bands
   without liquid in all empty wells

Connecting and Starting Power Supply

1. Select a good location for the placement and use of the equipment where it will not easily be knocked over or bumped.
2. Do not place the unit on grounding points or conductors (near sinks, metal plates or other metal objects or surfaces).
3. Place the unit on the laboratory bench in such a way that the power supply (on/off switch) is easy to reach. The power supply should be to the side of the electrophoresis unit not behind it.
4. Make sure adequate clearance is established around the apparatus. Never allow the leads to dangle below the laboratory bench.

	5. Inspect the equipment and wiring before use. Look for signs of damage. Do not use worn or frayed high voltage leads (i.e. exposed wires, cracks or breaks, etc.) 6. Check the electrophoresis chamber for cracks or leaks and missing lids. 7. Before placing any chemical into the electrophoresis chamber check to see that the chamber has a design that prevents buffer contacting the power supply. The electrodes should not be in contact with the chemical solution when the power supply is on. 8. The lid of the chamber should fit tight onto the electrodes preventing buffer contact when running electrophoresis. 9. Ensure that all switches, lights, and all safety interlock features are in proper working condition and that "Danger-High Voltage" warning signs are in place on the power supply and buffer tanks. 10. Report any and all machine deficiencies prior to use and use only apparatuses in proper working condition. 11. Locate all emergency power source shut-off locations. 12. Carefully place the lid on the top of the gel box, ensure it is completely pressed down and level. **MAKE SURE RED WIRES CONNECT TO RED PORT ON BOX AND BLACK WIRES TO BLACK PORT.** 13. Make sure that the power is off before connecting the electrical leads. Connect the wires to the correct color terminals on the power supply. 14. Plug in the power supply 15. Set to desired voltage. 16. Always think and look before touching any part of the apparatus. Never touch any part of the apparatus while the power is "ON", not even the plastic parts. 17. NEVER open the gel box lid or reach inside the gel box until the power has been turned off. Do not rely on safety interlocks, as they may fail. Do not override safety devices. 18. Ensure bubbles start to form on both sides of the gel box where the buffer reservoirs are. 19. After # mins the gel should be complete check that samples have migrated by looking through the lid. 20. Turn off power supply. 21. Disconnect power supply from outlet.
7.	**TRANSPORT, RECEIVING AND STORAGE REQUIREMENTS** *Describe transport, receiving and storage requirements. Include secondary containment, transport devices (carts, carriers, etc.), segregation requirements, any special temperature or atmospheric requirements, and container compatibility requirements. Information may be included in Section 6.*

Chemical name	*Storage location/requirement*
Glacial acetic acid	Storage location Keep separated from strong oxidants, inorganic acids, bases hydroxyl compounds and ethylene glycol. Store away from sources of ignition and heat.
Na2EDTA	Storage location Keep separated from oxidants. Liquids and solids must stored separately.
Boric acid	Storage location Keep separated from strong oxidizing agents, strong bases, potassium, and acid anhydrides. Liquids and solids must be stored separately.
Ethidium bromide	Storage location Liquids and solids must be stored separately. Store away from strong oxidizing agents.
Loading dye (methylene blue, bromophenol blue, cresol red, etc.)	Storage location Liquids and solids must be stored separately.

8. **WASTE DISPOSAL**

Type of waste generated by this procedure/process (*check all that apply*):
☐Solid ☐Liquid

Waste hazard determination (*check all that apply*):

Type of Waste	*Hazard Determination*
Solid	☐Flammable ☐Oxidizer ☐Corrossive ☐Reactive ☐Toxic ☐Biological ☐Radioactive
Liquid	☐Flammable ☐Oxidizer ☐Corrossive ☐Reactive ☐Toxic ☐Biological ☐Radioactive

Expected waste generation per experiemntal run (mass/volume): Click or tap here to enter text.

- Satellite Accumulation Area is located XX
- Ethidium Bromide gels can be easily de-stained in the laboratory by simply placing the gels in a DI-water bath for 15 minutes and gently agitating the gels. This

	eliminates the need to collect the gels as a hazardous waste, and labs are highly encouraged to do so. • Concentrated stock solutions (10 mg/L or greater) containing Ethidium Bromide and agarose gels stained with Ethidium Bromide that have not been diluted as described above must be handled as Hazardous Waste. • All other materials involved with the processing or use of Ethidium Bromide (including diluted solutions, running buffers, de-stained Agarose gels, rinsates, and solid materials which have contacted only Ethidium Bromide diluted solutions) may be safely disposed of down sink drains (if liquid waste) or as Clean Lab Ware (if solid waste) **as long as no other hazardous materials are also present in the waste**. • Several other dyes common in the electrophoresis process (typically in gel loading buffers) include xylene cyanol, methylene blue, bromophenol blue, and cresol red. Unused portions of these dyes should be turned over to EHS for disposal as hazardous waste, while dilute aqueous solutions containing the dyes may be disposed of down sink drains **as long as no other hazardous materials are also present in the waste**. • Pipette tips and tubes in contact with the sample must be disposed as biohazardous waste.
9.	**EMERGENCY PROCEDURES** *Indicate how spills, personnel exposure/injury, and other accidents should be handled and by whom.*
	Incidents of concern would include exposure to ethidium bromide or boric acid (or any Particularly Hazardous Chemical), burns from hot agarose, exposure to UV light, electrical shock, or a large spill of concentrated ethidium bromide or boric acid containing solution (or any other Particularly Hazardous Chemical > 1 L) **Life-threatening emergencies** (*fire, explosion, large-scale spill or release*) • **ACTIVATE THE BUILDING'S FIRE ALARM SYSTEM IF THE SPILL REPRESENTS A THREAT TO HUMAN LIFE OR MAY CAUSE A FIRE OR EXPLOSION.** • Notify all persons in the workspace that a spill has occurred and evacuate all personnel from the workspace to a safe location. • Isolate the work space to prevent inadvertent entry: lock any access doors, place signs on doors reading "DO NOT ENTER-CHEMICAL SPILL" • **Call EHS at 392-8400 for clean-up assistance. If the emergency occurs outside of normal work hours, contact the University Police Department at 392-1111.** **Personnel Exposure (refer to SDS):** • BBP Needlestick & BBP Splash Exposures: call Needlestick Hotline at 1-866-477-6824 (OUCH). Immediately after evaluation/treatment, employees should contact AmeriSys at 1-800-455-2079. Report incident to EHS. • Chemical contact with skin: Remove contaminated clothing and jewelry and wash affected area with plenty of soap and water. The emergency shower is located XX.

Obtain medical attention if skin irritation or rash occur or if you feel unwell. Report to EHS.

- Chemical contact with eyes: Remove contact lenses. Flush with water for 15 min in eyewash station located XX. Irrigate eyes thoroughly while lifting eyelids. Seek medical advice if necessary. Report to EHS.
- Chemical ingestion: Rinse mouth with water (do not swallow). Never make an unconscious person vomit or drink fluids. Call poison control center and obtain medical assistance if you feel unwell. Report to EHS.
- Chemical inhalation: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a poison control center and obtain medical assistance if you feel unwell. Report to EHS.

For minor burns:

- Cool the burn. Hold the burned area under cool (not cold) running water or apply a cool, wet compress until the pain eases.
- Remove rings or other tight items from the burned area. Try to do this quickly and gently, before the area swells.
- Don't break blisters. Fluid-filled blisters protect against infection. If a blister breaks, clean the area with water (mild soap is optional).
- Apply burn cream. Once a burn is completely cooled, apply burn cream found in the first aid kit.
- Report to EHS

Source: https://www.mayoclinic.org/first-aid/first-aid-burns/basics/art-20056649

For major burns, call 911.

In Case of Electric Shock:

Call 911 or your local emergency number if the injured person experiences:

- Severe burns
- Confusion
- Difficulty breathing
- Heart rhythm problems (arrhythmias)
- Cardiac arrest
- Muscle pain and contractions
- Seizures
- Loss of consciousness

Take these actions immediately while waiting for medical help:

- Turn off the source of electricity, if possible. Do not attempt to touch the victim until they have been isolated from the source of electricity. If you are unable to get to the power supply, move the source away from you and the person, using a dry, nonconducting object made of cardboard, plastic or wood.
- Begin CPR if the person shows no signs of circulation, such as breathing, coughing or movement.
- Try to prevent the injured person from becoming chilled.

- Cover any burned areas with a sterile gauze bandage, if available, or a clean cloth. Don't use a blanket or towel, because loose fibers can stick to the burns.
- Report to EHS

Source: https://www.mayoclinic.org/first-aid/first-aid-electrical-shock/basics/art-20056695

In the event of a spill that can be cleaned up by local personnel:

- Notify personnel in the area and restrict access. Eliminate all sources of ignition.
- Review the SDS for the spilled material to determine the appropriate level of protection. Minimum protection should include gloves, safety glasses and lab coat.
- Wearing appropriate personal protection equipment, clean up spill using the lab's spill kit located at XX. Collect spill cleanup materials in a tightly closed container and label appropriately as hazardous waste. Wipe area with soap and water.

Decontamination of EtBr (≥ 10 mg/L):

- Using the proper PPE, prepare decontamination solution by adding 20mL of 50% hypophosphorous acid to a solution of 2g of sodium nitrite in 300mL of water.
- Soak paper towels in freshly prepared decontamination solution.
- Scrub the contaminated surface with the soaked paper towels.
- Using a UV light, check the area to ensure that all the ethidium bromide has been removed. Wear UV-blocking eyewear, full-face shield, long-sleeve protective clothing and gloves. Repeat decontamination procedure as necessary.
- Dispose of all contaminated towels, pads and other debris as hazardous waste.

Decontamination of diluted EtBr (< 10 mg/L):

- Using proper PPE, soak the spill up with paper towels or some other absorbent.
- Once the area is dry, spray the affected area with detergent and then scrub the area with a steel wool or brillo pad for several minutes.
- Using paper towels dry up the area and then wipe the area down with absorbents dipped in tap water. Repeat this process until the area is clean.
- Using a UV light, check the area to ensure that all the ethidium bromide has been removed. Wear UV-blocking eyewear, full-face shield, long-sleeve protective clothing and gloves. Repeat decontamination procedure as necessary.
- Dispose of towels, pads and other debris as non-regulated waste.

Emergency contact numbers:

Lab manager	xxx-xxx-xxxx
Building Manager	xxx-xxx-xxxx
Principal Investigator	xxx-xxx-xxxx
Poison Control Center	800-222-1222
Emergency	911
EHS	352-392-1591